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INFLUENCE OF CONCENTRATION OF PULLULAN COATINGS ONTO BARRIER PROPERTIES FOR FOOD PACKAGING

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Abstract

Nowadays society is every time more concerned about the environment. This has a clear impact in the industry, now that the consumers require sustainable products and eco-friendly. The use of biopolymers is one of the most promising strategies toward an optimized use of traditional packaging materials (e.g., oil-based plastics) without impairing the goal of extending shelf life. Among other food packaging materials, pullulan is attracting much attention due to its unique features.

This thesis aims to prove if pullulan, which is a polysaccharide polymer, eco-friendly, applied as coatings on polyethylene films, improves the functional properties of interest for the food packaging industry and in which concentration gives the highest performance.

In this document, you will find the different processes and analysis used to arrive at the conclusion that there is still a path to go through until use pullulan as active packaging, so it is needed to find a pullulan based formula that stabilizes it avoiding the fragility of this coating. These conclusions are based on the results of several analyses-experiments as FTIR (Fourier Transform Infrared Spectroscopy), goniometry, Oxygen barrier test, antimicrobial etc.

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1. Glossary

PET: Polyethylene terephthalate is the most common thermoplastic polymer resin of the polyester family. Consists of polymerized units of the monomer ethylene terephthalate, with repeating ($C_{10}H_8O_4$) units.

PP: Known as polypropylene, is a thermoplastic polymer used in a wide variety of applications. An addition polymer made from the monomer propylene.

Exopolysaccharide: Natural polymers of high molecular weight secreted by microorganisms into their environment.[1] These establish the functional and structural integrity of biofilms, and are considered the fundamental component that determines the physiochemical properties of a biofilm.[2]

2. Preface

This Document will analyze through specific analysis if different concentrations of pullulan coatings can bring better barrier properties for food packaging. It is proved that pullulan used as active packaging can help prevent the deterioration of foods by retarding dehydration, suppressing respiration, improving textural quality, allowing the retention of volatile flavor compounds, and reducing microbial growth.[3] It will also be examined if additional layer of antimicrobial polymer chitosan can improve material properties from antimicrobial point of view.

2.1. Origin of the idea

Food packaging continues to be one of the most important and innovative areas in development of new processes and products. Due to its importance it is widely used around the world in a massive way. At this time, most of the packaging is made using oil-based plastics which are proved to be harmful for the environment. Besides requiring protection, specific physical-chemical properties and attractive esthetical look, advanced food packaging materials function as a preservation system. Recently, R&D in food packaging materials is directed to develop bioactive, biodegradable, "intelligent", human and environmentally friendly packaging materials used in combination with vacuum treatment, atmosphere modification, freshness and time-temperature indicators, oxygen and leak indicators, colour measurement sensors, ethanol emitters, ethylene, O2 or CO2 absorbers, etc. and specific - biological packaging providing antimicrobial and antioxidant functionalities[11]. The origin of this thesis is to find a way to introduce bio polymers into this packaging chain, giving better properties and an eco-friendly side. The coating on conventional polyethylene foils will be prepared using macromolecular solution of polysaccharide Pullulan and Chitosan.

2.2. Motivation

The motivation to do this project is to introduce the coating based on the bio polymers as active packaging material concept. Among classical active substrates chemical antimicrobials, organic acids, e.g. benzoic acids, parabens, sorbates, sorbic, propionic, acetic and lactic acid are most frequently used as food preservatives, food contact substances and food contact material sanitizers.[15] However, consumers are increasingly aware with unhealthy side effects of chemicals (i.e. parabens, benzoic acids, nitrites, etc.) in food products. The changes in retailing practices (such as market globalization resulting in longer food transport routes) and the fact that industry must follow strict EU packaging guidelines and regulations, act as a driving force for R&D of advanced bioactive packaging materials. Examples for natural biodegradable and bioactive substances are less employed polysaccharides and their derivates. Among the various polysaccharide products, pullulan and chitosan are very interesting. Among pullulan properties the stability of its aqueous solutions over a broad range of pH, the low viscosity in comparison with other polysaccharides and the good oxygen barrier properties of films and coatings derivatives, show this material as a good candidate to manufacture new bio films.[7]

One of the most popular amino polysaccharide is chitosan obtained by alkaline deacetylation of chitin. Chitosan's positive charge, the degree of N-deacetylation, the mean polymerization degree and the nature of chemical modifications are the properties which strongly influence its antimicrobial effectiveness (Kumar, 2000). Chitosan has been approved as food ingredient from FDA recently; therefore, the use of chitosan for new product development as well as natural antimicrobial agents would become more popular. In food products, chitosan offers a wide range of applications, e.g. including preservation of food from microbial deterioration, formation of edible-biodegradable films, coagulation of proteins and lipids from waste water, enhancing of gelation in surimi and fishery products and clarification/deacidification of fruit juice. [8]

Several publications may be found for use of pullulan and chitosan as coating in packaging applications. However, to the best of our knowledge the combination of both as coating for conventional polyethylene was not research in the same manner as in this presented work.

Finally, to set a base into the development of food packaging composite material research and understand the processes and analysis which are used to determine the conclusions.

3. Introduction

Recently, biodegradable packaging material has become an essential part of the global food packaging market aiding to the ever increasing consumer awareness and importance of eco-friendly substitutes. The demand for biodegradable packaging is increasing and will continue to increase as the companies utilize packaging like a medium to protect their products and promote the safety of the environment.

The European packaging industry has a market value of about 80 billion EUR (Global Packaging Alliance 2011) and accounts for about 40 % of the global packaging market. The future trend is oriented toward growing markets on a global scale. The development of high-performance renewable materials is one important factor for sustainable growth of the packaging industry. The technologies to enhance sustainability in food processing are among others sustainable packaging and sensor technology and thus contribute to food safety and food security. Major manufacturers of packaging are now looking to differentiate their products from those of their competitors by providing the best possible functional biodegradable packaging products as per consumer demands. To be competitive it is important that new bio-based packaging solutions are multifunctional, economically viable and can be easily incorporated in present industrial manufacturing processes. Sufficient competiveness can only be realized if the EU food industry speeds up the pace of innovation.[16]

Over recent decades, much research on the development of innovative food packaging materials has been carried out with a view to combating pathogens, reducing spoilage and waste, optimizing process efficiency, reducing the need for chemical preservatives, improving the functionality of foods, and improving the nutritional and sensorial properties of food responding to the demands of the different consumer niches and markets, also in terms of affordability.[17]

However, few of this investigations were economically, eco and health friendly, thus it

is still a great challenge to be involved into study of new concept of active packaging material. Pullulan has emerged as one of the biopolymers with the greatest projection over the last years due to its properties as a coating for food packaging. Packaging can also block food and air direct contact, such as O2, N2 and CO2 in the air and other gases will accelerate the food in the protein, vitamins and other physical and chemical reactions. Many foods, especially fruit and vegetable products in the humidity or temperature are too high conditions, leading to deterioration or decay.[18]

Regarding active packaging concept development chitosan as antimicrobial aminobiopolymers obtained from chitin. The low production costs and biocompatibility make chitosan an attractive material for food processing, biomedical applications and water purification, just to mention a few. Chitosan has been approved as food ingredient from FDA recently; therefore, the use of chitosan for new product development as well as natural antimicrobial agents would become more popular. In food products, chitosan offers a wide range of applications, e.g. including preservation of food from microbial deterioration, formation of edible-biodegradable films, coagulation of proteins and lipids from waste water, enhancing of gelation in surimi and fishery products and clarification/deacidification of fruit juice. Beside several direct applications in food products, chitosan also exhibits the potential for use as food supplements with anti-cholester-olemic, anti-ulcer, anti-uremic and anti-tumour effects[12].

Due to its great properties, chitosan may be an ideal potential substance for polyethylene coatings for active packaging concept development. Due to its bacteriostatic function influences the elongation of the lag phase and, consequently, reduces the growth rate of microorganisms in order to extend shelf - life and to maintain product quality and safety[12].

Although there are several contributions based on the use of combination of chitosan with other antimicrobial/antioxidant properties for food preservation there has been no work on the use of complementary synergistic formulations of chitosan and pullulan.

Moreover, most of the research is limited to edible films and not to layer-by-layer coatings.

This thesis will be focused in determinate which is the best concentration of pullulan, among three levels (10%, 20% and 30%), that improve the barrier properties of food packaging as polyethylene. As additional layer chitosan solution will be adsorbed in order to introduce antimicrobial activity. The concentration of chitosan will be 1%.

The properties of functionalized foils will be determined by the following analysis:

Gravimetry: determination of the amount of coating printed on polyethylene film.

Goniometry: to measure the degree which the coating is hydrophilic or hydrophobic.

FTIR: To see the functional groups of the coating.

Standard test for oxygen permeability and antimicrobial testing of foils.

Desorption: check how stable is the coating into aqueous environments.

Antimicrobial test: To check the antimicrobial properties of the coating.

The scope of the thesis is to obtain the tendency of the concentration of pullulan and combination of pullulan with chitosan which allows a better performance of active packaging.

4. Food packaging and main problems

Packaging not only protects of food quality and safety, but also brings damage on the resources and the environment, and even leads to serious ecological problems.

Developed countries have been aware of the seriousness of the problem of packaging for environmental pollution since 1960s. The direct or indirect impacts of packaging on the environment include soil degradation, water pollution, and the sharp reduction of scarce resources such as forests, solid waste pollution and toxic chemical pollution. It seriously affected the sustainable development of resources and environment.[15]

The protective function of packaging for food is of paramount importance. In particular, agricultural products because of its own physical characteristics determine its natural vulnerability in the circulation process, external pressure or vibration during transport will cause the surface wear and tear, and even change its internal physical properties, thereby speeding up the deterioration of food speed.

External environmental factors include: gas; light; temperature and humidity. Appropriate packaging to a certain extent can reduce these injuries. Maintain the original nature of food, thereby enhancing the consumer's desire to buy. Simultaneously, packaging to a certain extent, play the role of isolation protection. The impact of light on food is the main food of certain substances (such as vitamins, proteins, fat etc.) in the role of light will accelerate the oxidation and decomposition, resulting in the loss of nutrients in food. Packaging can also block food and air direct contact, such as O2, N2 and CO2 in the air and other gases will accelerate the food in the protein, vitamins and other physical and chemical reactions. Many foods, especially fruit and vegetable products in the humidity or temperature are too high conditions, leading to deterioration or decay.

One of the main food substrates, requiring biologically active packaging, is also meat. It is prone to both microbial and oxidative spoilage, so various antimicrobial agents may be incorporated in the packaging system to preserve meat safety and quality.[14]

With the consumer demand for microwave food, snack foods and frozen food another convenience food is increasing, the demand for new concept of active packaging development is huge. That is why is needed to upgrade active packaging concept that is consumer and environmentally friendly. One of the interesting way is introduction of bio polymers (as additives or coatings) to this sector.

4.1. What is pullulan?

Pullulan is one of the biopolymers that have attracted much attention over recent years due to its peculiar characteristics. This non-ionic exopolysaccharide is obtained from the fermentation medium of the fungus-like yeast Aureobasidium pullulans (originally called Pullularia pullulans) under limiting conditions (e.g., nitrogen), with media composition and culture conditions highly affecting the final yield.[4] Pullulan is generally marketed as a white to off-white dry powder.[5] It is non-toxic, non-mutagenic, non-carcinogenic, and edible.[6] It is tasteless, odorless, and highly soluble in both cold and hot water and in dilute alkali, though it is insoluble in alcohol and other organic solvents except dimethylsulfoxide and formamide.[5] It has a good mechanical strength and other functional properties such as film and fiber formability, adhesiveness and enzymatically mediated degradability. [7, 8]

Other interesting properties of this exopolysaccharide concern the stability of its aqueous solutions over a broad range of pH, the low viscosity in comparison with other polysaccharides and the good oxygen barrier properties of films and coatings derivatives, which show this material as a good candidate to manufacture new bio films.[9]

Pullulan as a biodegradable and biocompatible biopolymer, it has achieved wide

regulatory acceptance with its proven safety record. In the United States, pullulan has “generally regarded as safe” status.[10]

Due to its peculiar characteristics, pullulan is extensively used in different sectors, the three main realms of application pertaining to the pharmaceutical, biomedical, and food fields.

In the area of the food industry, early applications of pullulan involved its use as a thickening, stabilizing, texturizing, and gelling agent, providing products with good sensory properties, extended shelf life, and easier processing.[13]

Early attempts to employ pullulan in the food packaging industry lagged behind the established use as a food additive (e.g., thickening agent, binder, stabilizer), the first works dating back to the beginning of the ’90s. At that time, it was understood that great benefits would have arisen from certain peculiar properties of pullulan, such as its high water solubility and the barrier property against oxygen and carbon dioxide. At the beginning, water-soluble edible films of pullulan were proposed as edible pouches for premeasured portions that could be gradually dissolved in water or in hot food. However, the first massive application of pullulan in food packaging can be considered its use as an edible coating, i.e., a relatively thin layer of material applied and formed directly on the surface of the food product, which can be eaten along with the product.[14]

However, the first massive application of pullulan in food packaging can be considered its use as an edible coating, i.e., a relatively thin layer of material applied and formed directly on the surface of the food product, which can be eaten along with the product.

4.2. What is Chitosan?

Among the various polysaccharide products, amino fucntional polysaccharides are the most promising as antimicrobial substances (Patton et al. 2006), usefull for many applications. Useful for many applications including packaging. These polysaccharides amino groups which interact with the cell surface of pathogen microorganisms and in this way destroy them by several possible mechanisms (Kumar, 2000). One of the most popular amino polysaccharide is chitosan obtained by alkaline deacetylation of chitin. Chitosan's positive charge, the degree of N-decetylation, the mean polymerization degree and the nature of chemical modifications are the properties which stronlgy influence its antimicrobial effectiveness (Kumar, 2000).

Chitosan has been approved as food ingredient from FDA recently; therefore, the use of chitosan for new product development as well as natural antimicrobial agents would become more popular. In food products, chitosan offers a wide range of applications, e.g. including preservation of food from microbial deterioration, formation of edible-biodegradable films, coagulation of proteins and lipids from waste water, enhancing of gelation in surimi and fishery products and clarification/deacidification of fruit juice. Beside several direct applications in food products, chitosan also exhibits the potential for use as food supplements with anti-cholester-olemic, anti-ulcer, anti-uremic and anti-tumor effects[12]. Also, it has been shown that chitosan can be functionalized with antioxidant molecules by using enzymes (Sousa et at., 2009). Some reports revealed the antimicrobial effect of chitosan used as coating agents in chilled pork and sausages. It is important to note, that most research on chitosan activity has been conducted in solution, less with chitosan films, which appears to be very promising. However, extrapolation to packaging applications as surface coating is not so trivial whilst understanding of adsorption/desorption phenomena is needed and precise surface characterization need to be done.

5. Structure of the Analysis-Experimental part

To determine if the coating has significant effects, it has been necessary to put in practice different analysis, which results will contribute to a scientific conclusion.

As all experiments, it is not possible to have ideal results. It will be a part of the results that respond to measurement error and to interaction with variables we cannot control as, air humidity, some evaporation while preparing the dissolutions and the stability of temperature in a narrow range.

In the first step coating solutions based on pullulan and chitosan will be prepared. Coatings on conventional polyethylene will be done by printing (rolling) procedure. Functionalized foils will be analyzed regarding:

- Gravimetric Measurements of sample mass.
- Goniometry Analysis.
- FTIR.
- Standard test for oxygen permeability and antimicrobial testing of foils.
- Desorption Test.
- Antimicrobial test.

The hypothesis of the thesis is that coatings of pullulan will improve the barrier properties (oxygen permeability will be reduced) and addition of chitosan onto foils will introduce antimicrobial efficiency. The combined concept could find the application as active packaging material.

5.1. Preparation of the coating's dissolutions

10%, 20% and 30% of pullulan solutions were performed in volume of 25ml.

All the dissolutions has been prepared in the same way. It has been mixed the solute and the dissolvent (water) for 3 hours between 40ºC and 50ºC with a cover to avoid evaporation. The conventional foils like and PE were used to be functionalized with established formulations.

The first dissolution was made of water and pure pullulan with the following concentrations:

Dissolution Number	Concentration of Pullulan
1	10%
2	20%
3	30%

Table 1 Dissolutions of pullulan.

Due to problems of barrier releases during the drying phase it has been prepared another concept to bring better performance of pullulan coatings in this phase. It's been added Glycerol proportionally to the concentration of pullulan. This new formula brought elasticity to the coating, therefore, fixed the barrier releases.

Figure 1 Coating releases

As it is shown at Figure 1, at high concentrations of pullulan with no addition of glycerol (30%) the coating was impossible to be fixed on the film.

Also for lower concentrations, the coating has lost elasticity after a period of time and it can be seen that it has released at is shown at the next picture.

Figure 2 coating releases at lower concentration.

The new approach is the following one:

Dissolution Number	**Concentration of Pullulan**	**Concentration of Glycerol**
1	10%	3%
2	20%	6%
3	30%	9%

Table 2 New approaches.

Figure 3 Pullulan in powder.

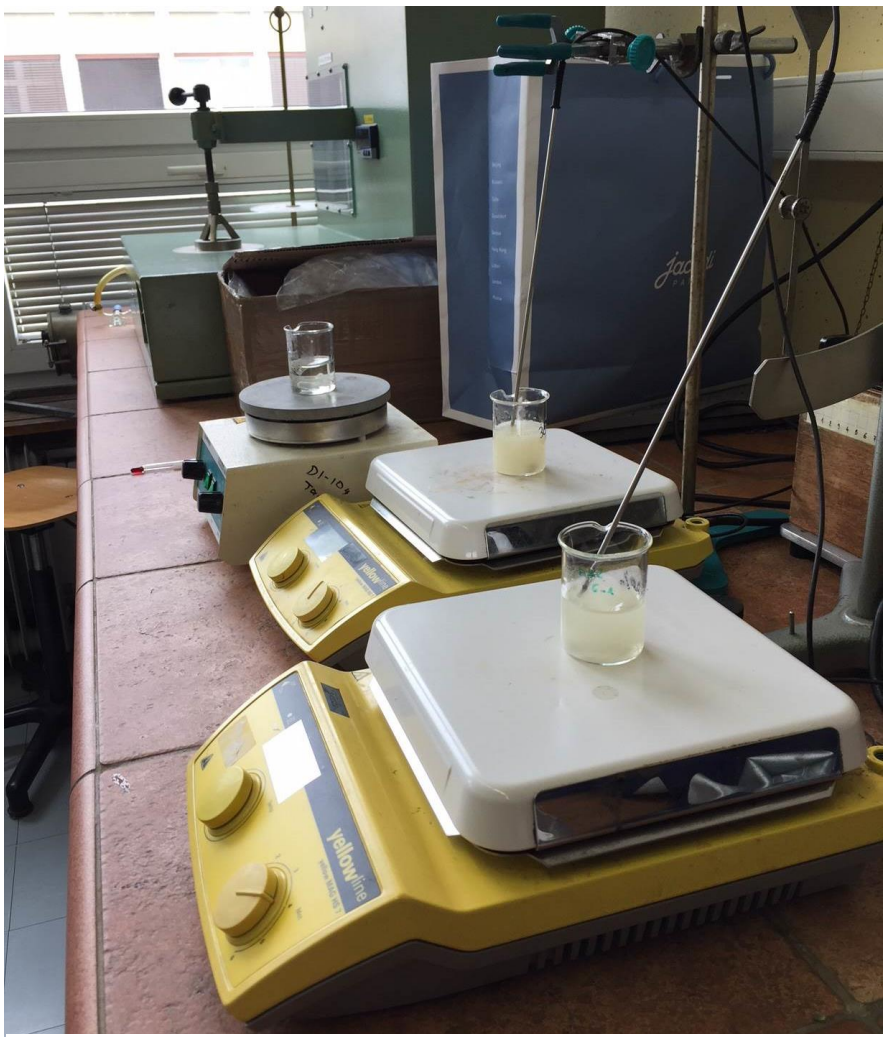

Figure 4 Preparation of the dissolution of Pullulan + Glycerol.

Moreover, in order to introduce antimicrobial properties a 1% Chitosan concentration acidic media solution was prepared and after printed on some samples of the dissolutions from table 2.

At the end there were 9 types of samples, those are shown at the next table:

Samples	Coating
1	10%Pullulan
2	20% Pullulan
3	30%Pullulan
4	10%Pullulan + 3%Glycerol
5	20%Pullulan + 6%Glycerol
6	30%Pullulan + 9%Glycerol
7	10%Pullulan + 3%Glycerol+1%Chitosan
8	20%Pullulan + 6%Glycerol+1%Chitosan
9	30%Pullulan + 9%Glycerol+1%Chitosan

Table 3 Summary of the samples.

5.2. Description of the Methods

- Gravimetric measurements of sample mass:

All the samples were weighed for the purpose of comparing their weights with untreated reference foils, which were previously cleaned, dried and cut to the size of the print. The final weight differences between the reference foils and applied foils

showed a successful application of the formulation onto the foil.

- Goniometry:

Contact angles were measured to estimate the surface of polymer with coating by goniometer DataPhysics, Germany. This is useful to determine surface characteristics of solid materials and their wettability by measuring the contact angle between the horizontal plane of the material and a liquid drop. The most commonly used method is to measure it directly using a drop of liquid resting on surface. Goniometer with SCA 20 software was used to measure the static contact angle at room temperature with Milli-Q water. A small drop (3 µl) of liquid (water) was carefully placed on the surface of the polymer film (plasma/non plasma). The static contact angle (SCA) measurement gives information on the hydrophilic/hydrophobic nature of the material. The water contact angles of hydrophilic materials are below 90 °, while they are above 90 ° for hydrophobic.

- Surface elemental composition of functionalized foils: ART FTIR Spectroscopy:

The ART FTIR spectra were recorded on a Perkin Elmer Spectrum GX NIR FT-Raman spectrometer. The ATR accessory contained a diamond crystal. All the spectra (16 scans at 4 cm-1 resolution, background and the sample spectra were obtained in the 400-4000 cm-1 wavenumber range) were recorded at room temperature. Spectra of samples were deconvoluted with smoothing filter and baseline corrected (automatic). To determinate FTIR, it has been cut pieces of samples and analyzed 5 times each on to avoid the possible no homogeneity of the coating's surface.

- Oxygen Permeability:

The oxygen permeability was determined using Oxygen Transmission Rate System PERME® OX2/230, Labthink Instruments Co., Ltd. PR China, by standard ASTM D3985. OTR (oxygen transmission rate) values and coefficient values are average result obtained by two testing of five measurements. All specimens were conditioned at 23°C and 50% relative humidity 24h prior testing (flux = 10ml/min). Thickness of PE and PP were measured with caliper at 5 different places.

- Desorption Test:

The determination of TOC is carried out in two steps: 1. disposing of the inorganic carbon (TIC) 2. Decomposition of the organic carbon (TOC) and detection of the carbon dioxide formed by means of an indicator. Range: 40–600 mg/L C, Factor: 0410. (-), Wavelength (HW = 5–12 nm): 585 nm, Decomposition time: 2 h, Decomposition temperature: 120 °C.

- Antimicrobial Test:

For antimicrobial test was used colorimetric methods, which could represent an alternative approach, using tetrazolium salts as indicators, since bacteria convert them to coloured formazan derivatives that can be quantified. While they are all good indicators of bacteria growth, difficulties arising because of autofluorescence, salt reduction and the antioxidant properties of plant products, especially for XTT (3′-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis (4-methoxy-6-nitro) benzenesulfonic acid hydrate), TTC (2,3,5-triphenyl tetrazolium chloride) and resazurin, make them less suitable indicators for MIC assay. The antimicrobial activity of extracts as the minimal inhibitory concentration (MIC) was tested for gram-positive (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli).

5.3. Printing of coating onto polyethylene film

In order to proceed to the different analysis it is needed to print the dissolution onto polyethylene films. To do it, it's been used a printing machine using the pressure of an iron cylinder attracted by a magnetic field to distribute homogenously through a membrane the dissolution on the film.

There has been 40 printings during the period of analysis. Some of them for repetition and the rest to print the different dissolutions prepared.

It can be seen during the practical work that the viscosity increases depending on the concentration of pullulan, this fact created some difficulties to print the most viscous dissolutions so the one which contains 30% in concentration of pullulan it's been printed without using the membrane. Also to ease the dissolution's flow through the membrane's pores and get better results, the viscosity has been decreased by increasing the temperature while printing.

Figure 5 Printing Machine used.

As it is said before, it was printed two different solutions, a pullulan one and also a pullulan and glycerol one. There was also an additional printing of chitosan on the solution with better performance (with glycerol). Chitosan was added on the film to see if it would bring more functional properties as antimicrobial efficiency.

5.4. Gravimetric Measurements of sample mass

All the samples were weighed for the purpose of comparing their weights with untreated reference foils, which were previously cleaned, dried and cut to the size of the print. The final weight differences (absolute dry samples) between the reference foils and applied foils showed a successful application of the formulation onto the foil.

The Gravimetric Analysis It's been helpful for two different things. First of all to see the factor of water remaining into the polyethylene film and second, to determine the quantity of material implementing the function as a coating.

To determinate the factor of water, it's been used a machine that warms up the polyethylene film up to 130ºC for 40-70 seconds (depending on the amount of plastic's mass) until the weight converges to a determinate final mass (with no water).

In this way the moisture content in polyethylene was calculated, On the base of this that gravimetric result was pointed out as mass difference for absolute dry sample.

Figure 6 Gravimetric Analysis tool.

Since it's been done several printings, this analysis was made in different times, one for the dissolution of just pullulan and the other one for the dissolutions pullulan + glycerol and pullulan + glycerol + a coating of chitosan.

The results obtained after 5 repetitions are the following ones:

Dissolution printed	**Water Factor**
Pullulan	-10,08%
Pullulan + Glycerol and Chitosan	-0,52%

Table 4 Water Factors.

As it's seen, there is a big difference with the water factor, this is probably because the first printing was made with polyethylene film in contact with the atmosphere for a longer time than the second film. At the end this results don't affect at the final mass deposited onto the film.

The next step it's been to weight the film, print the coating on it and weight the film again. To do it, it's been used a high precision scale.

Figure 7 High precision Scale.

The results Obtained are the following ones:

Sample	Film	Mass before	Pullulan Concentration	Mass after Printing coating	Coating Mass
1	Polyethylene	2,0754g	10%	2,1554g	0,08g
2	Polyethylene	2,1972g	10%	2,2841g	0,0869g

3	Polyethylene	2,3001g	20%	2,5001g	0,2g
4	Polyethylene	2,4579g	20%	2,6451g	0,1872g
5	Polyethylene	2,3493g	30%	2,6751g	0,3258g
6	Polyethylene	2,1996g	30%	2,5112g	0,3116g

Table 5 difference of mass before and after printing the coating with no glycerol.

Sample	**Film**	**Mass before**	**[Pullulan]**	**[Glycerol]**	**Mass after**	**Added Chitosan**	**Coating mass**
1	Polyethylene	3.0059g	10%	3%	3,0283g	No	0,0224g
2	Polyethylene	2,9369g	10%	3%	2,9582g	No	0,0213g
3	Polyethylene	2,8415g	10%	3%	2,8833g	Yes	0,0418g
4	Polyethylene	2,8915g	10%	3%	2,9489g	Yes	0,0574g
5	Polyethylene	3,7094g	20%	6%	3,7911g	No	0,0817g
6	Polyethylene	3,3964g	20%	6%	3,4925g	No	0,0961g
7	Polyethylene	3,2382g	20%	6%	3,3939g	Yes	0,1557g
8	Polyethylene	3,2547g	20%	6%	3,4109g	Yes	0,1562g
9	Polyethylene	2,6303g	30%	9%	2,7278g	No	0,0975g
10	Polyethylene	2,9049g	30%	9%	2,992g	No	0,0871g
11	Polyethylene	3,1606g	30%	9%	3,2981g	Yes	0,1375g
12	Polyethylene	2,7192g	30%	9%	2,8015g	Yes	0,0823g

Table 6 difference of mass before and after printing the coating with glycerol and chitosan.

The mass on the tables is calculated for dry samples and it can be seen that for all samples mass increased which somehow pointed out the application of the coatings was successful. It can also be seen that when chitosan was printed onto different pullulan coated polyethylene the mass increases in higher extend.

5.5. FTIR

The Fourier Transform infrared spectroscopy is an analysis that allows to determinate which are the functional groups that we can find on the surface of coated materials. This is very useful to see if the products applied are really attached onto surface.

Figure 8 FTIR tool used for the analysis.

The following graphs show the more remarkable wavenumbers of the spectrum. The ones with a number are the functional groups that are considered a contribution from the dissolutions printed as a coating.

To have a better understanding of the functional groups that are discussed in this part of the thesis, the next table shows the main IR frequencies of interest for that analysis:

Simplified Table of Main IR Frequencies

Wave number, cm^{-1}	Functional Group	Peak Description
3300 – 3600	O-H (alcohol)	Strong and broad
2500 – 3000 can reach	O-H (carboxylic acids)	Very broad (over ~ 500 cm^{-1}), often looks like distorted baseline, above 3000 cm^{-1}.
3200 – 3500	N-H	Doublet in case of NH_2 group of a primary amine or amide
3300	C≡C—H terminal alkyne	Usually sharp and strong
3000 - 3100	C=C—H alkene or arene	Often weak, overlaps with CH alkane absorption
2800 – 3000	C-H (sp^3 carbon)	Strong, broad and multi-banded
2250 - 2220	C≡N	Medium intensity
2100 - 2260	C≡C alkyne	Medium intensity for terminal alkynes, very weak for internal
1680 – 1820	C=O (amides, ketones, aldehydes carboxylic acid, esters)	Very strong; lower frequency for amides and when C=O is conjugated
1600 – 1650	C=C alkene, aromatic ring	Check to see if you have C-H unsaturated >3000 cm^{-1}(if not, it's completely substituted)
~ 1600	$-NH_2$ (bending) 1° amines and amides	Only if you have corresponding N-H peak at 3200-3500 cm^{-1} (this peak may be mistaken for C=C otherwise)
1200	Ar-O	Strong (look for =C-H & C=C first)
1050-1150	C-O	
690 and 750	phenyl group	Strong (look for =C-H & C=C first)

Table 7 main IR frequencies of interest

As it is seen, in all the spectrums we superpose pure pullulan, and polyethylene to have a reference of their main functional groups. The FT-IR spectrum of PE (red line, Fig.9) shows characteristic PE signals at following wave numbers 2914 cm^{-1}, 2847 cm^{-1}, 1470 cm^{-1} and 718cm^{-1}. FT-IR spectrum of pullulan (pink line, Fig. 9) shows typical signals in the range 3309 cm^{-1}, which correspond to vibration of OH group and the signals in the area around 988 cm^{-1}.

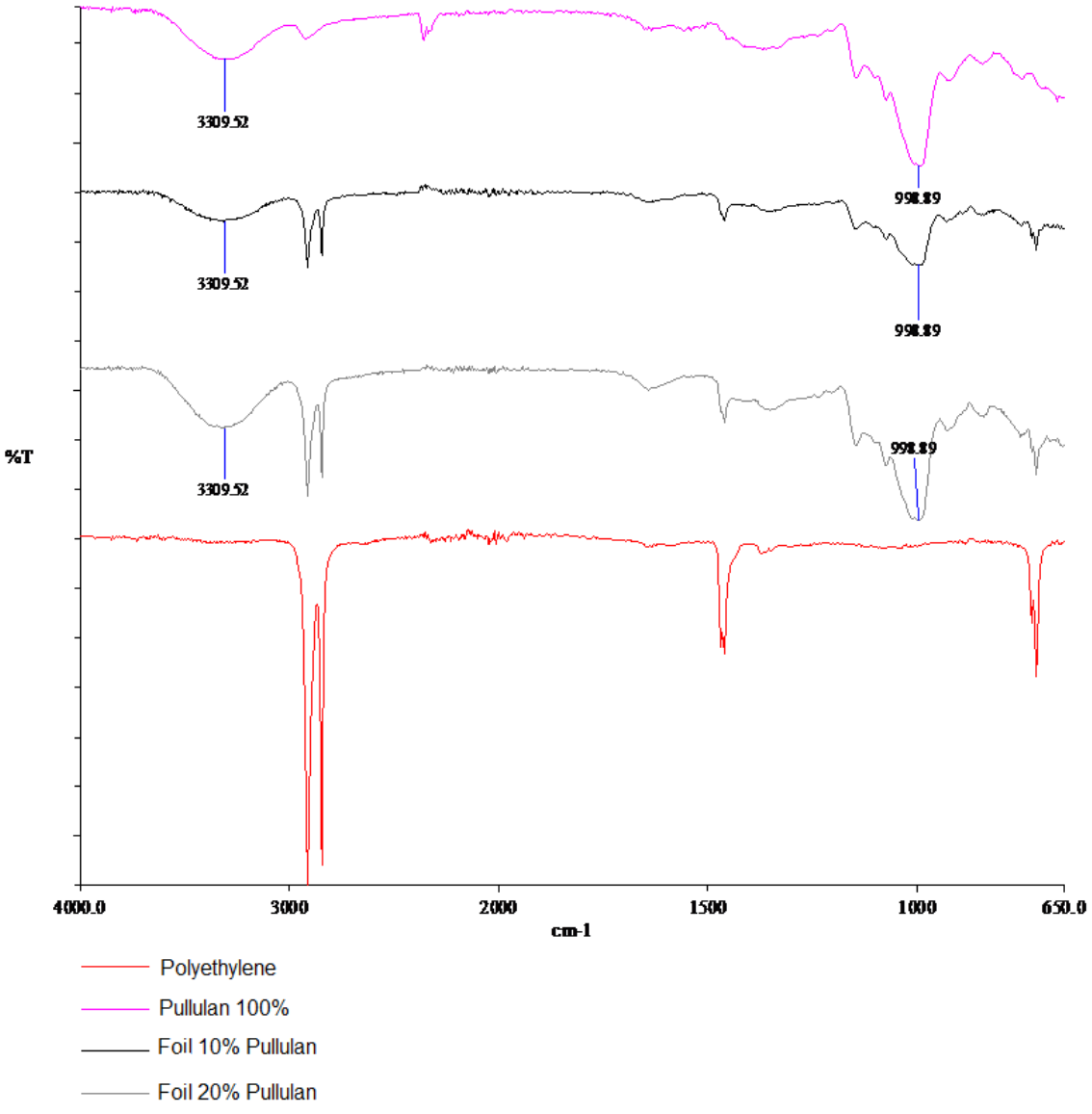

Figure 9 FT-IR spectra for the foil coating with pullulan solution no glycerol.

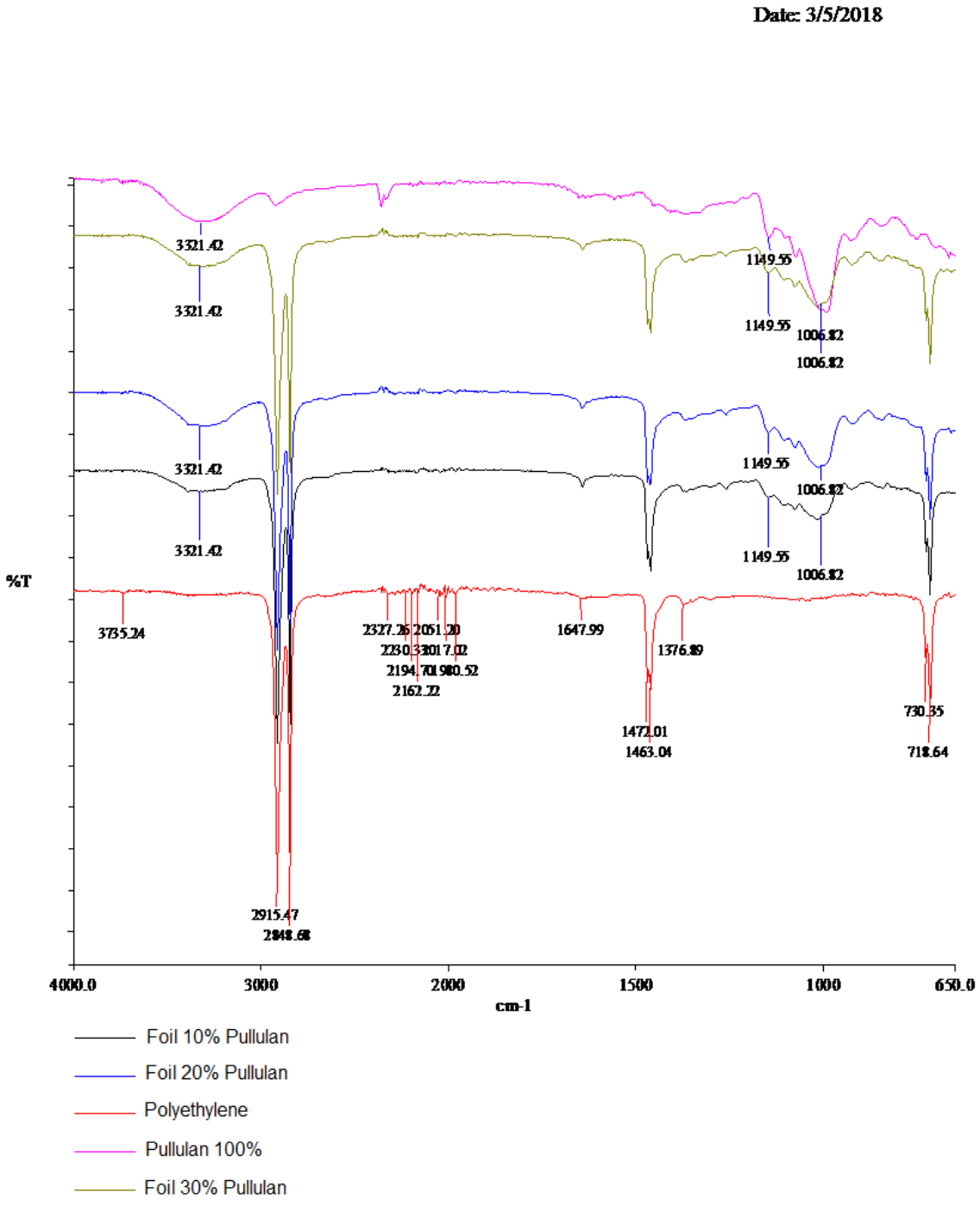

Figure 10 Frequency spectrum from the coating with glycerol.

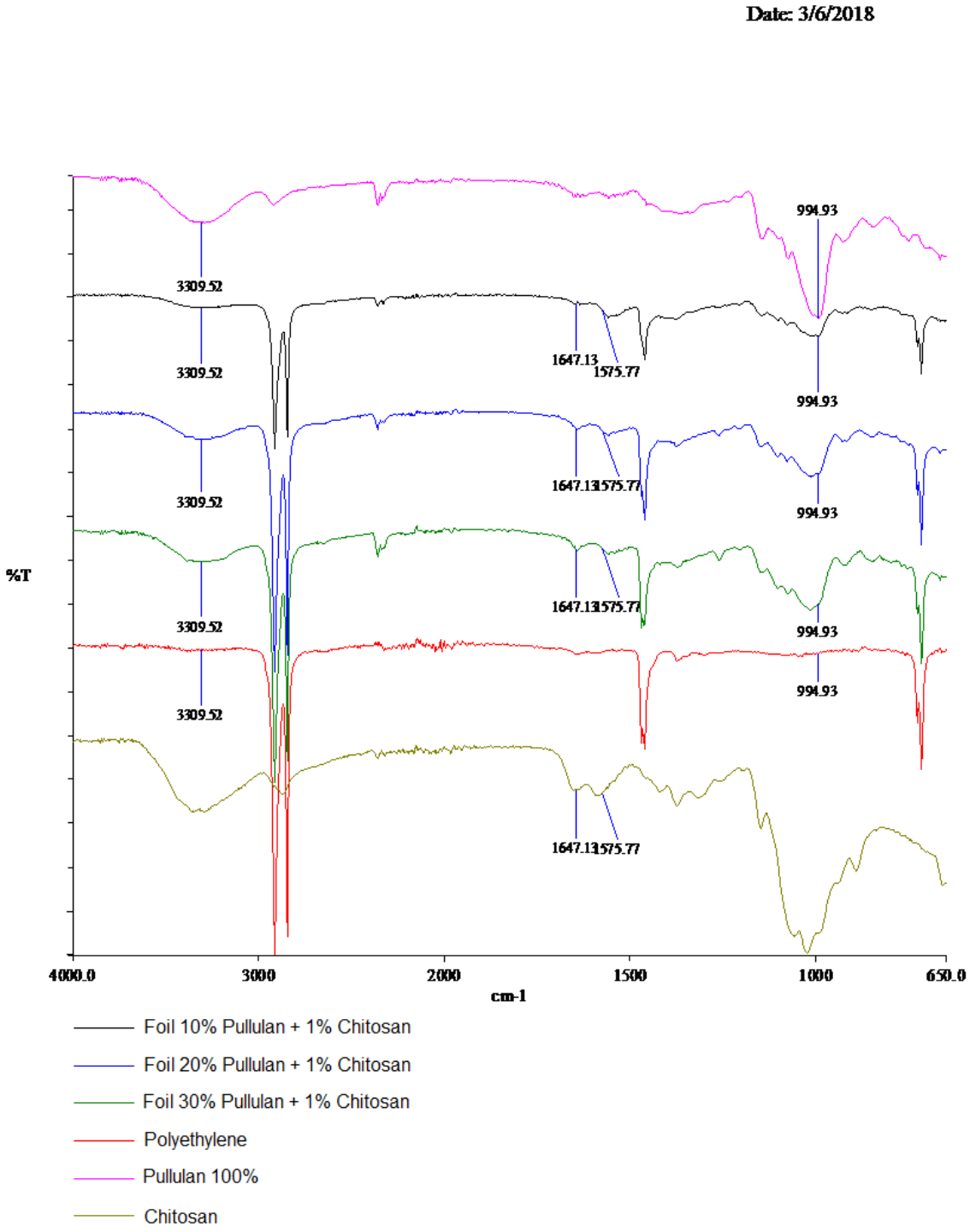

Figure 11 Frequency spectrum of dissolution with glycerol and chitosan.

The figure 9 is the spectrum of the Samples 1 and 2 from Table 3. In this case was not possible to determinate the spectrum of the 30% Foil (sample 3) because of the coating's detachments. FT-IR spectra of PE foil coated with 10 % (green line, Fig. 9) and 20 % (grey line, Fig. 9) solution of pullulan shows typical signals for pullulan. Signals at wavenumber 3309 cm^{-1}, and 988 cm^{-1} are more intensive for the PE foil coated with 20 % solution of pullulan.

The Figure 10 are spectra of references PE foil and pullulan and PE foil treated with 10 %, 20 % and 30 % solution of pullulan and glycerol (samples 4,5 and 6). FT-IR spectra of PE foil coated with 10 % (black line, Fig. 10), 20 % (blue line, Fig. 10) and 30 % (green line, Fig. 10) solution of pullulan and glycerol show typical signals for pullulan. Signals at wavenumber 3309 cm^{-1}, and 988 cm^{-1} are more intensive for the PE foil coated with 30 % solution of pullulan. Glycerol does not have influence on FT-IR spectra of treated foil.

FT-IR spectra treated foil don't show the difference to compere of the dissolution with glycerol. In this one, we can also see the fingerprint of pullulan on the coated samples and the higher peaks depending on the concentration of Pullulan.

Figure 11 presented references PE foil (red line, Fig. 11), pullulan (pink line, Fig. 11) and chitosan (light green line, Fig. 11), and PE foil treated with 10 %, 20 % and 30 % solution of pullulan and 1 % solution of chitosan (samples 7, 8 and 9).

The FT-IR spectrum of the chitosan (light green line, Fig. 11) showed typical peaks at 1649 and 1585 cm^{-1}. These two wavenumbers are assigned to the carbonyl stretching vibration (amide I), and the N-H bending vibration (amide II) of a primary amino group, respectively.

FT-IR spectra of PE foil coated with 10 % (black line, Fig. 11), 20 % (blue line, Fig. 11) and 30 % (green line, Fig. 11) solution of pullulan and 1 % solution of chitosan show typical signals for pullulan (signals at 3309 and 998 cm^{-1}) and chitosan (signals

at 1649 and 1585 cm^{-1}. Signals at wavenumber 3309 cm^{-1}, and 988 cm^{-1} are more intensive for the PE foil coated with 30 % solution of pullulan.

5.6. Goniometry analysis

The Goniometry analysis it is needed to determinate if the coating is hydrophilic or hydrophobic. This plays an important role for food packaging now that when there is temperature changing, if the coating is hydrophilic the droplets of water created are bigger causing a bad shelf life of the food. Reduction of the contact angle is of great importance for practical use, as the hydrophilic surface of the foils reduces the potential process of the foil condense dew in contact with the food which worsen the packaging conditions.

For this analysis is being used a sophisticated machine with a high-resolution camera. The machine leaves a little drop of water on the coating and then the camera defines the angle between the contact and the horizontal plane.

Figure 12 Contact angle tool.

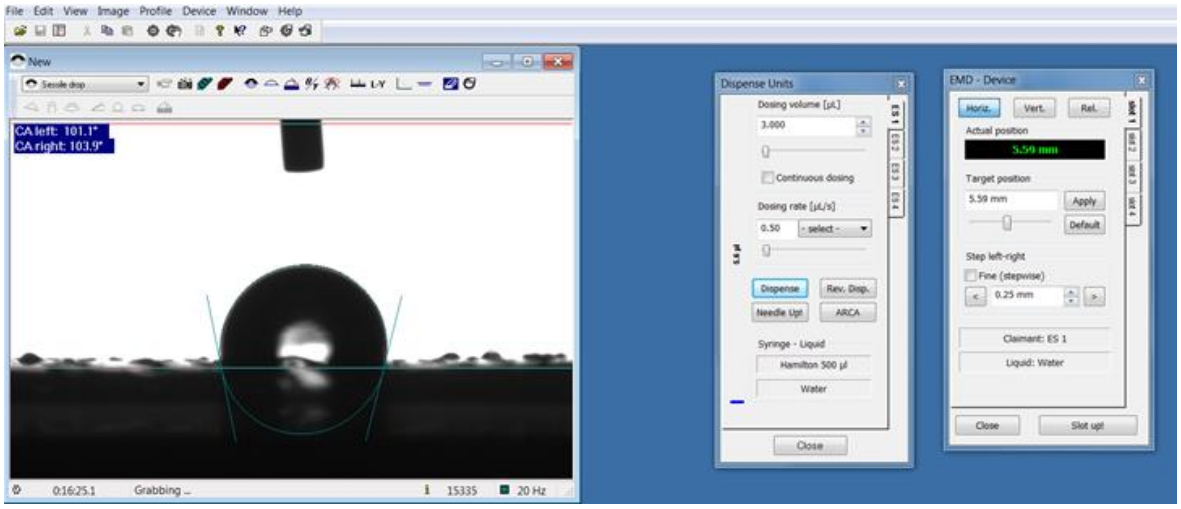

Figure 13 Screenshot of the procedure of measuring the contact angle.

The results obtained are at the Following ones:

Measurement : CA-measurement
Device : DataPhysics OCA - Series
Customer : DataPhysics
Sample : Not Defined
Operator : User
Remarks : DataPhysics

Run-No	CA(M)[°]		IFT[mN/m]		Err[µm]	Vol[µL]
1	94.76	0.00	37.30	431.84		
2	98.74	0.00	30.67	419.86		
3	105.37	0.00	35.76	242.31		
4	100.97	0.00	56.19	347.31		
5	101.50	0.00	40.74	399.24		
6	96.01	0.00	47.48	370.95		

Table 8 Results 10% Pullulan coating.

Measurement : CA-measurement
Device : DataPhysics OCA - Series
Customer : DataPhysics
Sample : Not Defined
Operator : User
Remarks : DataPhysics

Run-No	CA(M)[°]		IFT[mN/m]		Err[µm]	Vol[µL]
1	93.71	0.00	66.93	387.44		
2	99.56	0.00	94.45	436.07		
3	94.52	0.00	90.25	460.24		
4	104.65	0.00	23.75	416.48		
5	105.09	0.00	31.46	396.36		
6	103.46	0.00	6.29	422.62		
7	99.85	0.00	75.78	420.89		

Table 9 Results 20% Pullulan coating.

Measurement : CA-measurement
Device : DataPhysics OCA - Series
Customer : DataPhysics
Sample : Not Defined
Operator : User
Remarks : DataPhysics

Run-No	CA(M)[°]		IFT[mN/m]		Err[µm]	Vol[µL]
1	99.03	0.00	53.21	478.78		
2	93.21	0.00	44.51	432.33		
3	90.24	0.00	29.07	384.14		
4	90.58	0.00	17.41	300.80		
5	91.36	0.00	27.67	400.69		
6	93.28	0.00	38.16	378.68		

Table 10 Results 30% Pullulan coating.

Measurement : CA-measurement
Device : DataPhysics OCA - Series
Customer : DataPhysics
Sample : Not Defined
Operator : User
Remarks : DataPhysics

Run-No	CA(M)[°]		IFT[mN/m]		Err[µm]	Vol[µL]
1	105.79	0.00	18.41	473.06		
2	98.08	0.00	22.06	398.96		
3	107.46	0.00	12.09	463.78		
4	99.73	0.00	12.41	424.12		
5	110.46	0.00	14.53	432.74		

Table 11 Results 10% + Chitosan coating.

Measurement : CA-measurement
Device : DataPhysics OCA - Series
Customer : DataPhysics
Sample : Not Defined
Operator : User
Remarks : DataPhysics

Run-No	CA(M)[°]		IFT[mN/m]		Err[µm]	Vol[µL]
1	104.64	0.00	12.14	475.49		
2	102.93	0.00	16.51	401.04		
3	104.89	0.00	18.04	494.56		
4	110.39	0.00	11.17	426.20		
5	106.50	0.00	37.24	420.39		

Table 12 Results 20% + Chitosan coating.

Measurement : CA-measurement
Device : DataPhysics OCA - Series
Customer : DataPhysics
Sample : Not Defined
Operator : User
Remarks : DataPhysics

Run-No	CA(M)[°]		IFT[mN/m]		Err[µm]	Vol[µL]
1	105.35	0.00	18.42	472.71		
2	108.58	0.00	41.22	433.93		
3	102.76	0.00	21.87	515.98		
4	101.25	0.00	92.32	410.48		
5	106.31	0.00	36.03	430.83		

Table 13 Results 30% + Chitosan coating.

Sample	Average CA(M) [°]
Polyethylene	104,5
10%Pullulan	99,56
10% Pullulan + chitosan	104,304
20%Pullulan	100,12
20% Pullulan + chitosan	105,87
30%Pullulan	92,95
30% Pullulan + chitosan	104,85

Table 14 Average CA for each simple.

The results Obtained show that some pullulan coating reduce the contact angle while addition of chitosan in general negligible increase a contact angle in comparison with polyethylene. The differences are however very small and no significant influence in pullulan concentration on contact angle may be seen. It could be said that the addition of chitosan as the second layer increases the contact angle around a 5% in comparison with pullulan coated samples.

5.7. Oxygen barrier test

Sample	OTR (cm3/m2d)	STDV OTR	COEFFICENT (cm3cm/cm2scmHg)	STDV COEFFICENT	Thickness (mm)
Polyethylene	3225,97	61,6	3,2334E-07	1,3707E-08	0,05
10%	2119,7	49,0	2,1624E-07	6,66133E-09	0,05
10%+Ch	2227,5	14,1	2,2842E-07	1,41067E-09	0,05
20%	2228,6	26,0	2,28302E-07	2,39792E-09	0,05
20%+Ch	2251,3	42,1	2,308E-07	4,32782E-09	0,05
30%	2596,8	70,6	2,6624E-07	7,2298E-09	0,05
30%+Ch	2419,4	15,2	2,48067E-07	1,51767E-09	0,05

Table 15 Results of Oxygen barrier test.

As it can be seen, the permeability increases along the concentration of Pullulan, so it does improve the oxygen barrier property. However, the application of chitosan as a second layer doesn't improve in a significant way the oxygen barrier property. The later may also be a consequence of non-homogeneous coatings.

5.8. Desorption Test

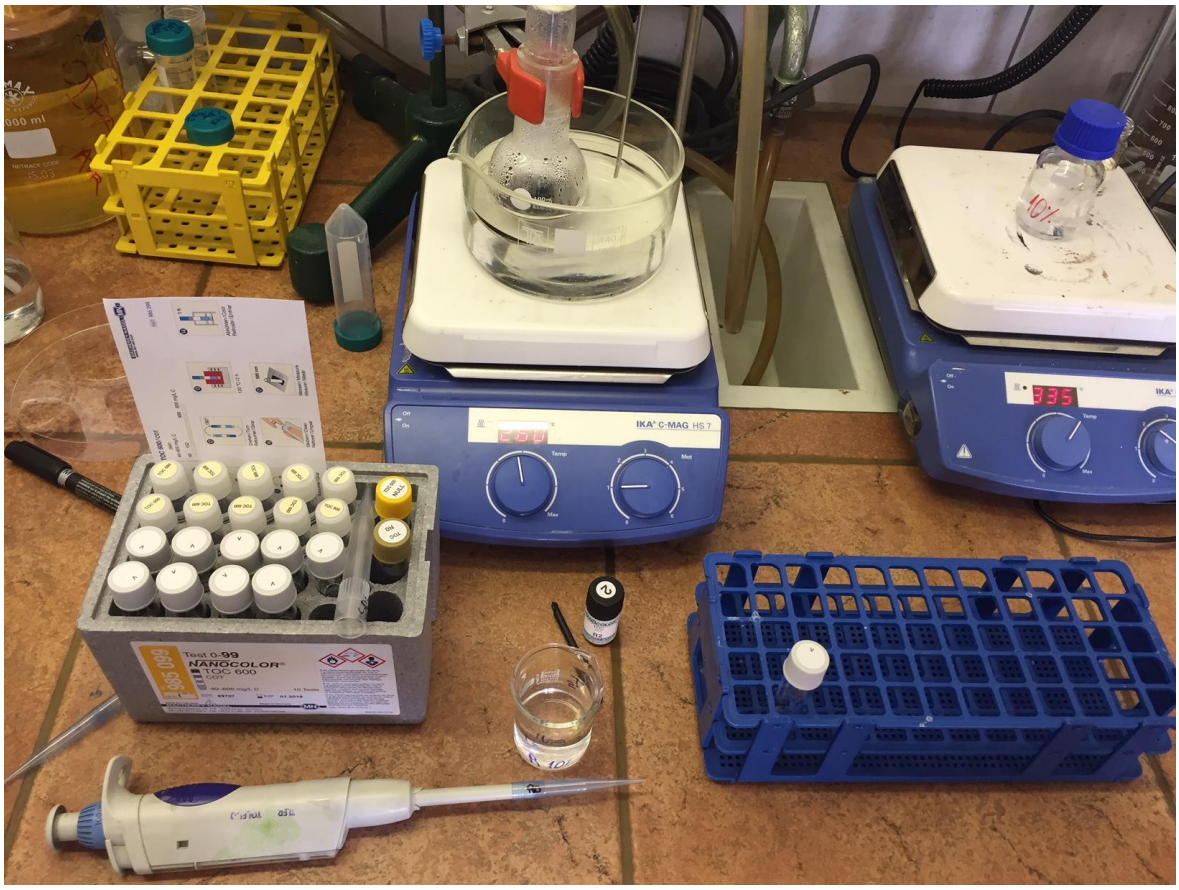

Figure 14 Desorption Analysis.

At the end, the result has been put into a spectrophotometer to calculate TOC on the base of measuring absorbance. More TOC determinated in desorption bath is indication of higher desorption of pullulan and chitosan. Both of these polymers possess in polymer backbone the C atoms. The results of TOC measurements of desorption bath are given in Table 15:

Sample	TOC
10% Pullulan	> 600 mg/l
10% Pullulan + Chitosan	< 40 mg/l
20% Pullulan	54 mg/l
20% Pullulan + Chitosan	< 40 mg/l
30% Pullulan	226 mg/l
30% Pullulan + Chitosan	98 mg/l

Table 16 Results of TOC desorption.

It can clearly be seen that when pullulan individually attached onto foils rather desorb from foil surface. The highest amount of TOC for desorbed pullulan was detected at lowest concentration of pullulan as adsorbate (10%).

This results may indicate that the lower concentration of pullulan the lower of OH groups are available which means he lower possibility of forming the physical interactions between pullulan and polyethylene. The later results into lower coating stability and higher desorption. In consequence, the total amount of organic carbon diluted into water is higher.

It can be also seen that chitosan acts as a good barrier for desorption of pullulan and chitosan. Obviously when chitosan applied as additional layer onto pullulan layer physical interaction between them and basic foils were extended and thus stability of coating improved. It seems that additional layer of chitosan onto polyethylene decrease the total desorption amount. All the samples with pullulan and chitosan together have a lower TOC than the ones that do not possess the Chitosan. If desorption will occur in big extended than TOC should be significantly higher whilst both pullulan and chitosan, that possess C atoms, are released from surface.

5.9. Antimicrobial Test

Staphyloccoccus Aureus

Antimicrobial properties against *S. aureus* are given in Table 17:

Sample	Cells number (log cfu/cm^2)	% of reductions
Control	4.75 ± 0.12	/
P-10% Antimicrobial	4.26 ± 0.21	10.31 ± 4.41
P-10% + CH Antimicrobial	2.76 ± 0.52	42.45 ± 10.28
P-20% Antimicrobial	4.64 ± 0.07	0
P-20% + CH Antimicrobial	4.32 ± 0.10	9.01 ± 2.17
P-30% Antimicrobial	4.01 ± 0.16	12.92 ± 4.05
P-30% + CH Antimicrobial	2.23 ± 0.81	52.95 ± 17.12

Table 17 Antimicrobial properties against S aureus results.

Polyethylene show no inhibition on *S.A.* It is clearly seen that attachment of pullulan onto foils do not introduce significant antimicrobial efficiency. Polyethylene coated by 10 and 30 % of pullulan show some small reduction. Additionally coated pullulan-polyethylene foils by chitosan showed the improvement of antimicrobial activity against *S.aureus*. The highest inhibition (63%) is determined by sample P-30% coated by 1% of chitosan solution. Obviously here the highest amount of chitosan was attached onto functionalized foils and thus more available amino groups for antimicrobial efficiency. It is known that higher amount of protonated amino groups increase antimicrobial activity.

Antimicrobial properties against fungi *A. flavusv* are given in Table 18.

Sample	Cells number(log cfu/cm^2)	% of reduction
Kontrola	2.52 ± 0.28	/
P-10% Antimicrobial	2.34 ± 0.31	0
P-10% + CH Antimicrobial	2.49 ± 0.15	0
P-20% Antimicrobial	2.24 ± 0.19	10.98 ± 7.48
P-20% + CH Antimicrobial	2.36 ± 0.07	6.02 ± 2.77
P-30% Antimicrobial	2.29 ± 0.32	0
P-30% + CH Antimicrobial	1.71 ± 0.16	31.99 ± 6.48

Table18 Antimicrobial properties against A. flavusv results

Polyethylene shows no inhibition on *A. flavus*. Fungi are much more resistant to antimicrobial agents. It could be that the antimicrobial properties of chitosan are antibacterial not antifungals. From our results it may be seen that only polyethylene foils coated with pullulan and then with 1% of chitosan improved in some extend (32%) the antimicrobial activity against *A. flavus*. Both microbial test show that sample P-30% + CH could with some improvement found the application for packagin

Conclusions

It is Obvious that packaging optimization and the reduction of oil based plastics for the food industry is condemned to decrease the next years in substitution of bio polymers more environmentally friendly. Active packaging is playing an important role in this global objective by apportions that definitely improve shelf life of food.

In this thesis it's been able to demonstrate that Pullulan can improve good barrier properties as oxygen permeability by itself, however chitosan decreases this same property so it should not be mixed for achieving an improvement of oxygen permeability.

Desorption properties have increased with higher concentrations of pullulan, and settled down with a good performance when used chitosan as a second layer.

It can be conclude that pullulan coatings have better barrier properties generally when used chitosan as a second layer but not in all the properties that we were looking for in the hypothesis. These two products could definitely, with a more extended research, be used as active packaging in the future. However there is still a long way to find ways to stabilize pullulan onto the coating in a way that allows to commercialize it. There is also a big handicap for pullulan which is the he price that makes impossible to use it in a large scale as industrial production.

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